

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (currently amended) A method to increase ~~for~~ RNA ~~or~~ polypeptide synthesis from a DNA template comprising: ~~the steps of~~

a) providing a cell-free system ~~enabling RNA or polypeptide synthesis from a DNA template, said~~

b) adding a DNA template comprising a strong bacterial promoter with at least one UP element[[;]] to said cell free system, and

~~b)~~ c) recovering said synthesized RNA ~~or polypeptide;~~

wherein characterized in that the ratio of an α subunit of RNA polymerase to other subunits concentration in said cell-free system ~~the concentration of α subunit of RNA polymerase, but not of other subunits,~~ is increased as compared to the conventional ratio of two α , one β , one β' and one σ in said cell-free system, comparing to its natural concentration existing in the cell-free system.

2. (currently amended) The method according to Claim 1, wherein said ~~system enabling RNA or polypeptide synthesis from a~~

~~DNA template is a~~ cell-free system ~~comprising~~ comprises a bacterial cell-free extract.

3. (currently amended) The method according to Claim 1 2, wherein the strong bacterial promoter ~~on the DNA template~~ includes a sequence from the *argC* gene promoter of *Bacillus stearothermophilus*, ~~preferably, the sequence from nucleotide -89 to +1, when the latter is the first nucleotide in mRNA of the argC gene.~~

4. (currently amended) The method according to Claim 2, wherein said cell-free system further comprises a purified thermostable RNA polymerase holoenzyme.

5. (original) The method according to Claim 4, wherein said thermostable RNA polymerase holoenzyme is from *Thermus thermophilus*.

6. (currently amended) The method according to Claim 2, wherein the concentration of said α subunit of RNA polymerase is increased by adding a purified α subunit of RNA polymerase to the bacterial cell-free extract.

7. (currently amended) The method according to Claim 6, wherein said purified α subunit is added to a final concentration ~~comprised~~ between 15 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$.

8. (currently amended) The method according to Claim 2
6, wherein the bacterial cell-free extract ~~extracts~~ is prepared
from cells overexpressing a gene encoding an α subunit of RNA
polymerase.

9. (currently amended) A method ~~for the production of~~
to increase the production of a protein from a DNA template in a
cell-free system ~~characterized in that it comprises the steps of~~
comprising:

a) providing in a reaction mixture, a bacterial cell-
free system ~~enabling the coupling of in vitro transcription of a~~
~~specific gene from a DNA template, and the corresponding protein~~
~~synthesis;~~

b) adding to the reaction mixture the DNA template
encoding a the desired protein and a purified α subunit of the a
RNA-polymerase; and[[,]]

~~e) optionally, adding a thermostable RNA polymerase,~~
~~and,~~

[[d)]]c) recovering the produced protein

wherein the DNA template comprises a strong bacterial
promoter with at least one UP element.

10. (currently amended) The method according to Claim
9, wherein said added RNA polymerase is a thermostable RNA
polymerase ~~is~~ from *T. thermophilus*.

11. (currently amended) The method according to Claim 9, wherein said purified α subunit is added to a final concentration ~~comprised~~ between 15 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$.

12. (previously presented) The method according to Claim 9, wherein a DNA-binding regulatory protein is further added to the reaction mixture at step (b).

13. (previously presented) The method according to Claim 9, wherein said DNA template comprises an amplification product of an Open Reading Frame encoding the desired protein.

14. (currently amended) The method according to Claim 13, wherein said DNA template further comprises an additional DNA fragment, which is at least 3 bp long, ~~preferably longer than 100 bp and more preferably longer than 200 bp,~~ located immediately downstream of the stop codon of said Open Reading Frame.

15. (original) The method according to Claim 13, wherein said DNA template further comprises an additional DNA fragment containing a transcriptional terminator.

16. (currently amended) The method according to Claim 15 ~~13~~, wherein said transcriptional terminator is a ~~the~~ T7 phage transcriptional terminator.

17-27. (canceled)

28. (new) The method according to Claim 9, wherein a thermostable RNA polymerase is further added in step b).

29. (new) The method according to Claim 3, wherein the promoter comprises a sequence from nucleotide at position -89 to nucleotide at position +1 of the *argC* gene promoter of *Bacillus stearothermophilus*, when position +1 is the first nucleotide in mRNA of the *argC* gene.

30. (new) The method according to Claim 9, wherein the strong bacterial promoter with at least one UP element is from the *argC* gene of *Bacillus stearothermophilus*.

31. (new) The method according to Claim 30, wherein the strong bacterial promoter includes sequence from at position nucleotide -89 to nucleotide at position +1 of the *argC* gene promoter of *Bacillus stearothermophilus*, when position +1 is the first nucleotide in mRNA of the *argC* gene.

32. (new) The method according to Claim 15, wherein said additional DNA fragment is longer than 100 bp.

33. (new) The method according to Claim 15, wherein said additional DNA fragment is longer than 200 bp.

34. (new) The method according to Claim 6, wherein said purified added α subunit of RNA polymerase is different from an α subunit present in the bacterial extract.

35. (new) The method according to Claim 34, wherein said purified added α subunit is from *E. coli*, *T. maritima* or *T. neapolitana*.

36. (new) The method according to Claim 1, wherein the UP element is a AT-rich region around 18-20 bp long.

37. (new) The method according to Claim 2, wherein said bacteria cell-free extract is from *E. coli* cells.

38. (new) The method according to Claim 37, wherein said *E. coli* cells are K12A19 cells having a *rna19 gdhA2 his-95 relA1 spoT1 metB1* genotype.

39. (new) The method according to Claim 6, wherein the purified added α subunit is purified from cells overexpressing a gene encoding an α subunit of RNA polymerase.

40. (new) A method for RNA or polypeptide synthesis from a DNA template comprising:

- a) providing a bacterial cell-free extract;
- b) adding a DNA template comprising a strong bacterial promoter with at least one UP element to said cell extract, and
- c) recovering said synthesized RNA or polypeptide;

wherein the ratio of an α subunit of RNA polymerase to other subunits concentration in said cell-free system is increased as compared to the conventional ratio of two α , one β ,

one β' and one σ , by adding in said bacterial cell free extract a purified α subunit of RNA polymerase prepared from cells overexpressing a gene encoding said α subunit of RNA polymerase.